LITTER RESPIRATION AND FUNGAL GROWTH UNDER LOW TEMPERATURE CONDITIONS

SUZANNE VISSER and D. PARKINSON

DEPARTMENT OF BIOLOGY - UNIVERSITY OF CALGARY
CALGARY, ALBERTA, CANADA

I. INTRODUCTION

Studies on the colonization of newly-fallen leaf litter by fungi have concentrated on the qualitative rather than the quantitative aspects. In fact there is a lack of studies concerning the amount of fungal colonization of such litter. MINDERMAN and DANIELS (1966), using a direct observation technique, studied the initial colonization of oak leaf litter by actinomycetes, bacteria and fungi. However, in their study regular sampling was not begun until approximately three weeks after leaf-fall. They indicated that bacterial numbers were extremely high immediately after the freshly fallen litter was moistened by rain and that fungal lengths were not considerable until three weeks after the leaf litter was moistened. WAID, PRESTON and HARRIS (1973) studied the fungal colonization of beech leaves, and attempted to distinguish between metabolically active mycelium and inactive or dead mycelium by using an autoradiographic technique. They observed that mycelial lengths reached a peak 21 days after leaves were placed in the sampling sites. However, their study was limited to leaf surfaces, and therefore excluded those hyphae actively growing within the leaves.

Fungal hyphae have the capability to penetrate substrates, and one would assume that, their growth form would allow them to colonize freshly fallen leaves rapidly under temperature and moisture conditions which are not limiting. VISSER (1971) showed that under conditions of high litter moisture content (70 % wet weight), hyphal lengths increased by approximately 4000 m.g. dry litter^{-1} within three weeks after leaves of *Populus tremuloides* fell to the forest floor. This would indicate a high ability of the fungi to colonize this type of leaf litter if moisture and temperature conditions are not extreme.

Because fungi can attain high mycelial lengths per gram dry substrate (and therefore considerable biomass) they can also play an important role in the immobilization of nutrient elements during the course of leaf litter decomposition. AUSMUS and WITKAMP (1974) have observed that in the 01 litter of a *Liriodendron tulipifera* forest, microbial immobilization of carbon can range from 0.8 to 29 % whilst that of nitrogen can reach a maximum of 79 % of the total nitrogen in the litter. They also indicated that potassium and phosphorus become limiting during spring, and thus they pointed out that these elemental limitations can adversely affect microbial growth and primary

production. Further details on elemental concentration by fungi (hyphae, rhizomorphs, fruit bodies, etc.) have been provided by the use of electron microprobe analysis (TODD et al., 1973) and have been discussed in a previous paper (PARKINSON and LOUSIER, 1974).

The aims of the study reported here were to attempt to determine the rate of colonization of freshly fallen leaves *(Populus tremuloides)* by fungi, to attempt estimation of the amount of mycelium containing protoplasm present on and in the leaf litter, and to obtain some data on the effects of environmental factors (such as moisture and temperature) on the rate of colonization. In addition, laboratory measurements were made of respiration rates of leaf litter samples at a series of temperatures at each sampling time, and an attempt was made to correlate respiration rate with mycelial content. From the mycelial data obtained, it was possible to estimate nutrient immobilization in the fungal component of the leaf litter biota.

II. METHODS

Details of the cool temperate *Populus tremuloides* woodland in which this study was conducted, together with litter input and decomposition rate data, have been given previously (PARKINSON and LOUSIER, 1974).

Leaves of *P. tremuloides* were collected at the time of natural leaf fall in aerial nets (1.5 m above the ground). The collected leaves were apportioned into 60 nylon mesh bags (10 mm mesh), with 20 leaves per bag. These litter bags were then placed on the surface of the litter layer in a 10 m x 6 m sampling area of the forest floor.

The litter bags were sampled at 11 times during a 186-day period between October and April, with particular attention being given to the first 33 days. At each sampling time 5 litter bags were taken at random and returned to the laboratory for immediate experimental treatment.

During the course of this study a continuous record of temperature at 0-1 cm in the litter layer of the forest floor was taken.

In the laboratory the leaf material from each litter bag was separately divided for the following studies :

i) Moisture content determination : weighed sub-samples were dried to constant weight at 80 °C and the moisture content calculated on a % wet weight basis.

ii) Measurement of litter respiration : oxygen uptake by aspen litter was measured in the conventional way using a Gilson respirometer with modified flasks which allowed entire leaves to be placed in the base of the flask. An equilibration period of 24 hours preceded measurements of oxygen uptake hourly for at least four hours at each of three temperatures (0,5 and 10°C).

At the completion of the sequence of respiration studies the leaf samples were removed from the flasks and their moisture contents determined (oven drying to constant weight at 80°C).

Respiration data for each sample at each temperature were then expressed in µl O_2 taken up per gram dry weight of leaf tissue per hour.

iii) Determination of amounts of mycelium : A 1 g sample of leaf material from each

litter bag was fixed (Bouin-Hollande) immediately on return to the laboratory, and subsequently the litter-agar film method (modified from the soil-agar film method of JONES and MOLLISON, 1948) was used to determine the amount of fungal hyphae in each litter sample.
In preparing the litter-agar films, maceration of the litter at 10,000 rpm for 3 min was found to be the optimal initial stage, with a final dilution of 1/200. The prepared litter-agar films were observed unstained but using phase contrast microscopy (20 fields on each of 10 films per litter sample being observed). In these observations, total lengths of fungal hyphae per microscope field were recorded as were lengths of mycelium containing protoplasm. This latter distinction was difficult for hyphae $\leqslant 2$ µm diameter.

III. RESULTS AND DISCUSSION

Figures 1 and 2 summarize the data obtained at each sampling time during this study on litter moisture and temperature, litter respiration at 0°C, total lengths of mycelium and lengths of mycelium containing protoplasm (Fig. 1 gives data for the period 12 Oct. - 18 Dec., Fig. 2 gives data for the period 18 Dec. - 16 April).
These data indicate that the initial colonization of freshly fallen litter by fungi is slow under the low ($<20\%$) moisture conditions found in the litter immediately following leaf fall. Increase in the litter moisture content (to 60 % wet weight) allows much more rapid fungal growth, i.e., there was an increase in mycelial length of 400 m g dry litter^{-1} within 3 days after an increase in moisture content (Fig. 1), even though the temperature at that time was falling.
During the winter period an increase in fungal colonization of the litter was still recorded, and it appears that temperature only becomes a limiting factor when it falls to about -3°C. The Student-Newman-Keuls test for grouped means in the analysis of variance demonstrated no significant differences (at the $P = 0.05$ level) both among mycelial lengths (both total mycelium and mycelium containing protoplasm) and litter respiration rates (measured in the laboratory at 0°C) measured in the December through March samples (Fig. 2).
The build-up of fungal populations in and on the freshly fallen litter has been demonstrated (in an as yet unpublished study) to be, in large measure, a result of colonization by fungi present in the forest floor rather than a result of proliferation of pre-existing leaf surface fungi.
The two figures given in Figure 2 for each measured parameter for the April sample indicate the 'patchy' nature of ice and snow thaw at that sampling time, i.e., some areas were completely snow-free and dry, whilst other areas, still under snow, were wet and colder. Therefore at this sampling time it was possible to record the effects of the rapidly changing environmental conditions occurring at snow melt, i.e., as temperature conditions rise under the moist sub-snow conditions mycelial growth increases significantly but after snow melt as litter rapidly dries (to a moisture content of $<20\%$ wet weight) a significant fall in mycelial lengths and litter respiration occurs. The fall in mycelial content at this time is probably due to a complex of factors - activity of

collembola was obviously very high at the time of snow melt and their activity as fungivores may be an important factor, also at this time of year diurnal temperature changes produce short-term freezing-thawing effects which may kill fungal hyphae (which may then be degraded by the increasing bacterial populations developing under the ameliorating environment of the litter).
Throughout the experimental period, a fairly high percentage of the mycelium containing protoplasm was recorded (i.e., $>50\%$ at all sampling times). Presumably this was because the study period was the time of initial colonization of litter by fungi, i.e., before the accumulation of empty hyphae had occured - as is the case in the fermentation layer of the organic horizon, where 20-30 % of the total mycelium measured contained protoplasm.

These data may be compared with those of NAGEL de BOOIS and JANSEN (1971) who estimated 79-82 % live mycelium in the litter layer and 15-20 % in the fermentation layer of an oak-beech forest. WAID, PRESTON and HARRIS (1973) also observed that a high percentage (76-97 %) of the total mycelium was active during the initial stages of the initial colonization of beech leaf surfaces by the fungi.
It is interesting to note the significant increase in mycelium with protoplasm following field moisture increase (Fig. 1, day 19), indicating active colonization of litter at this time of falling temperature. Following this burst of fungal activity the amount of protoplasm-filled mycelium stabilizes to 50-60 % total mycelium over the winter period (Fig. 2), and then exhibits the increase at snow melt referred to earlier.
Correlation coefficients were calculated for total mycelium, mycelium with protoplasm, respiration at 0,5 and 10°C, and moisture, and were found highly significant (at $P < 0.001$ level). However, field temperature correlated in a significant negative fashion with the above-mentioned variables. These correlation data are given in Table 1. Without having measured litter respiration in the 0 to –4°C range (i.e., ambient temperatures in the litter during the Dec. - Mar. period), it is difficult to discuss the field significance of the temperature correlation data.

With the development of microbial populations in leaf litter there is a corresponding tie-up of nutrient elements in microbial tissue. Tables 2 and 3 indicate the calculated nutrient immobilization in the fungal hyghae measured in this study. These data indicate that during the early phase of decomposition studied here, a peak of nutrient immobilization was reached in the period of snow-melt (186 days after leaf fall) when 0.6, 1.3, 1.1, 0.8, 0.45 and 0.16 % of the total litter content of C, N, P, Ca, Mg and K respectively are immobilized in the fungal component of the litter biota.

The fungal biomass figures (Table 2), when taken in comparison with comparable data on bacterial biomass (LOUSIER and ELLIOTT, 1974), indicate the relative importance of fungi in the initial stages of colonization (and decomposition) of leaf litter of *P. tremuloides,* i.e., three weeks after leaf fall bacterial biomass in the freshly fallen litter was 0.12 mg m^{-2}, whereas that of the fungi was 146.8 mg m^{-2}. However, direct comparison of these data is dangerous because the bacterial biomass figures were calculated from dilution plate counts and thus may represent a considerable underestimate, whereas the fungal biomass figures were calculated from direct observations.

ACKNOWLEDGEMENTS

Thanks are due to the National Research Council for an operating grant which allowed this study, to M. Mitchell and A. Carter for meteorological data, and to J. Bissett for statistical analysis of the data.

IV. REFERENCES

AUSMUS, B.S. and M. WITKAMP. (1974) – Litter and soil microbial dynamics in a deciduous forest stand. Publication No. 582, Environmental Sciences Div., Oak Ridge National Laboratories.

JONES, P.C.T. and J.E. MOLLISON. (1948) – A technique for the quantitative estimation of soil microorganisms. J. Gen. Microbiol. **2** : 54-69.

MINDERMAN, G. and L. DANIELS. (1967) - Colonization of newly-fallen leaves by microorganisms. In Progress in Soil Biology, O. Graff and J.E. Satchell (eds.), North Holland Publ. Co. pp. 3-9.

LOUSIER, J.D. and M.J. ELLIOTT. (1975) - Colonization of deciduous leaf litter by testate amoebae (Rhizopoda : Testacea) and bacteria. This symposium.

NAGEL DE BOOIS, H.M. and E. JANSEN. (1971) – The growth of fungal mycelium in forest soil layers. Rev. Ecol. Biol. Sol. T. VIII, **4** : 509-520.

PARKINSON, D. and J.D. LOUSIER. (1974) – Litter decomposition in a cool temperate woodland. This symposium.

TODD, R.L., K. CROMACK and J.C. STORMER, Jr. (1973) – Chemical exploration by electron probe microanalysis of decomposer organisms. Nature **243** (5409) : 544-546.

VISSER, S. (1971) – Ecology of fungi from aspen litter. M.Sc. Thesis. University of Calgary.

WAID, J.S., K.J. PRESTON and P.J. HARRIS. (1973) – Autoradiographic techniques to detect active microbial cells in natural habitats. In Modern Methods in the Study of Microbial Ecology, Th. Rosswall (ed.), Bull. Ecol. Res. Comm. (Stockholm) **17** : 317-322.

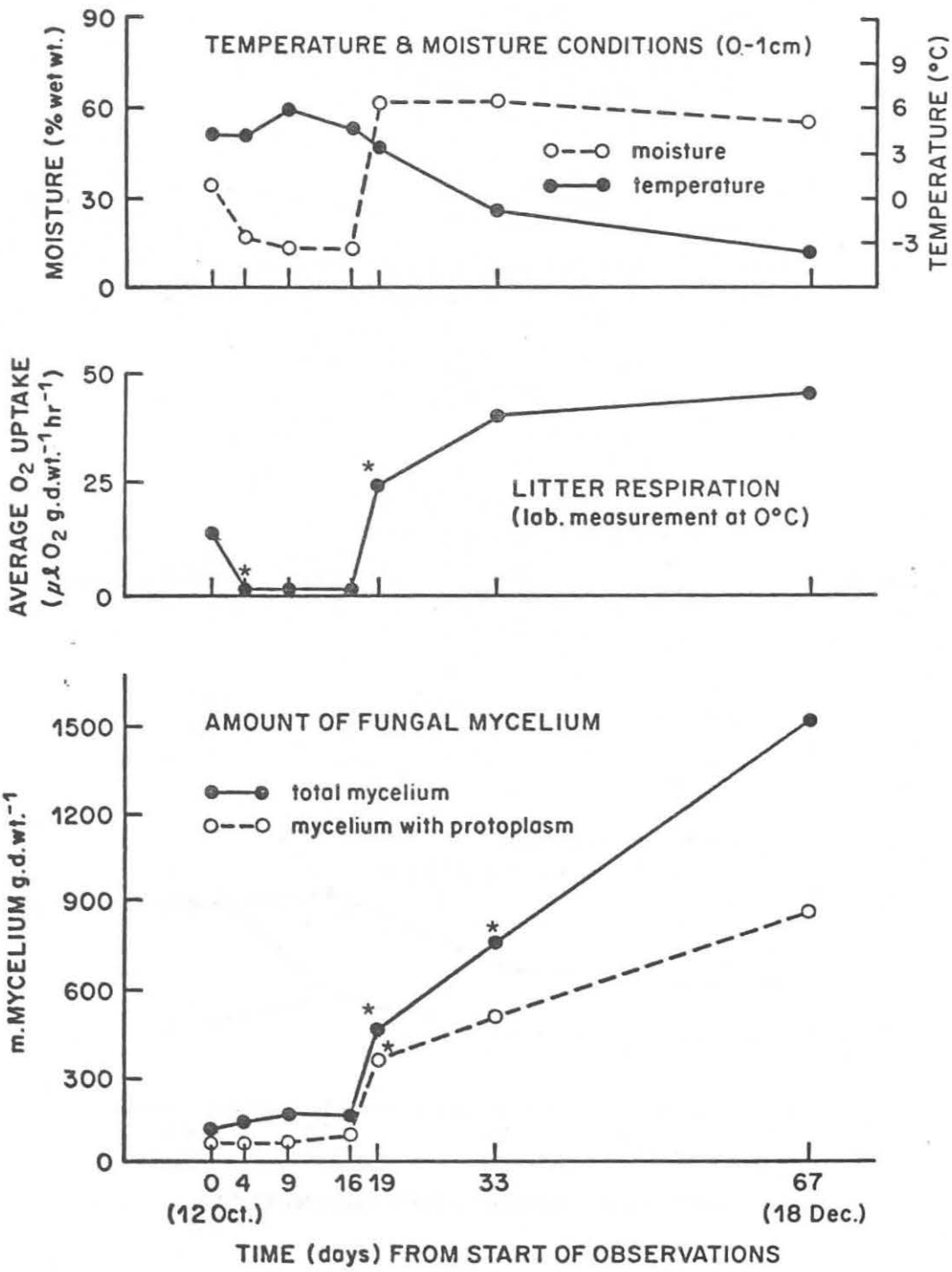

Figure 1. Changes in mycelial content, respiration rate, temperature and moisture conditions of the leaf litter during the first 67 days of observations (Oct. - Dec.)

* Indicates a significant difference ($P = .05$) from value recorded at previous sampling time.

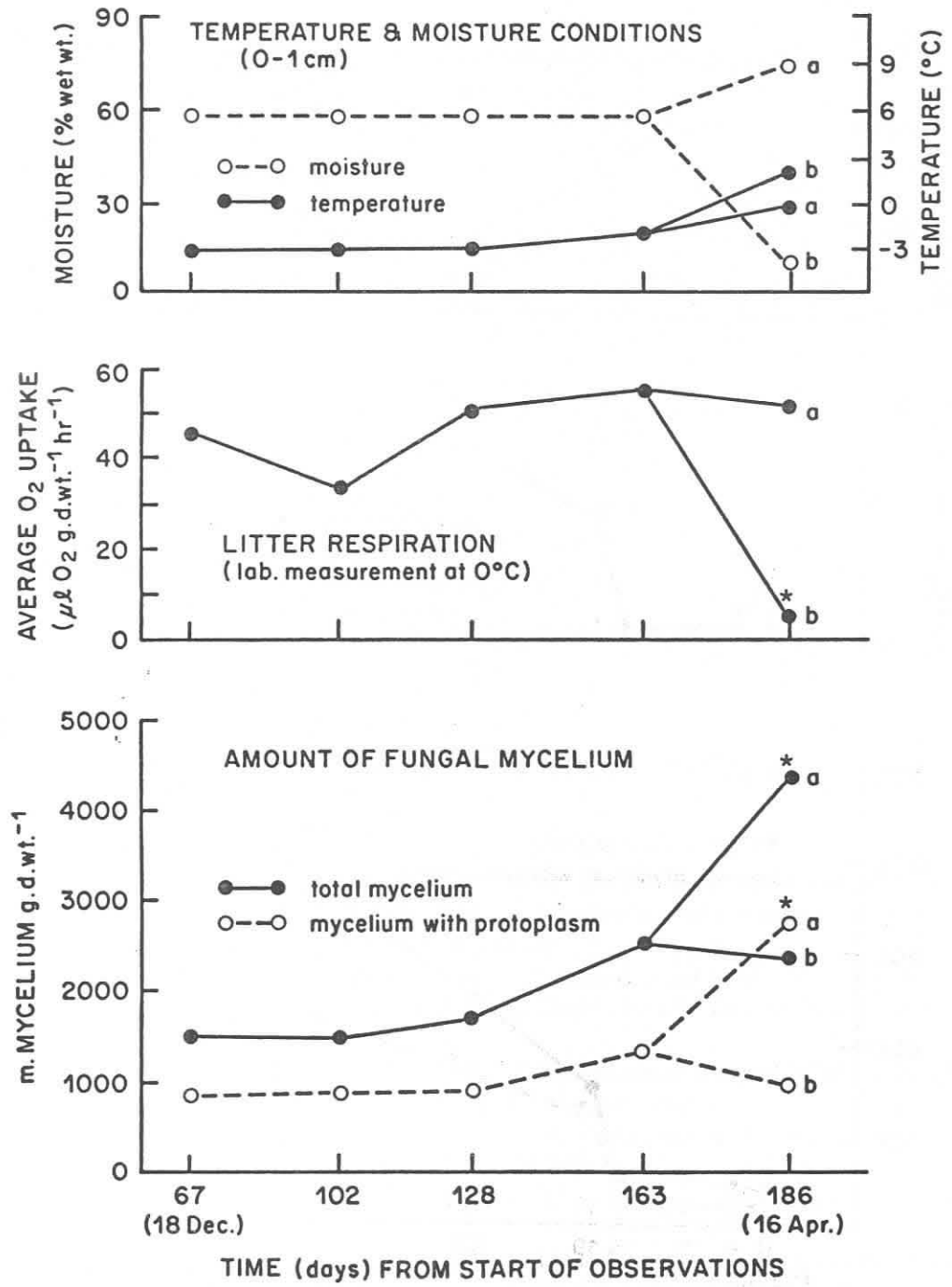

Figure 2. Changes in mycelial content, respiration rate, temperature and moisture conditions of the leaf litter from 67 - 186 days following the beginning of observations (Dec. - Apr.)

a : wet sample
b : dry sample

* Indicates a significant difference (P = .05) from value recorded at previous sampling time.

Table 1. Pearson correlation coefficients for total mycelial lengths, mycelium containing protoplasm, respiration at 10°C, field temperature and moisture.

	Total mycelium	Mycelium with protoplasm	Respiration at 10°C *	Field temperature	Moisture
Total mycelium	1.0000	.9646	.6379	-.5851	.5231
Mycelium with protoplasm	.9646	1.0000	.6149	-.5492	.5644
Respiration at 10°C *	.6379	.6149	1.0000	-.7990	.8522
Field temperature	-.5851	-.5492	-.7990	1.0000	-.7426
Moisture	.5231	.5644	.8522	-.7426	1.0000

All correlations significant, $P < .001$

* The correlation coefficients for the above variables were also highly significant for respiration measured at 0°C and 5°C.

Table 2. Carbon and nitrogen* immobilized in the fungal biomass at each sampling time.

Days from start of study	Month	Fungal biomass (mg d. wt. m^{-2}) **	Carbon (mg m^{-2} in fungal biomass	Nitrogen (mg m^{-2} in fungal biomass
0	Oct.	37.4	16.83	1.05
4	Oct.	43.2	19.44	1.21
9	Oct.	50.7	22.82	1.42
16	Oct.	50.7	22.82	1.42
19	Oct.	146.8	66.06	4.11
33	Nov.	241.9	108.86	6.77
67	Dec.	485.3	218.39	13.59
102	Jan.	493.2	221.94	13.81
128	Feb.	563.1	253.40	15.77
163	Mar.	807.5	363.38	22.61
186 (during snow-melt)	Apr.	1420.7	639.32	39.78
186 (after snow-melt)	Apr.	785.6	353.52	22.00

* Concentrations of carbon and nitrogen in dry fungal mycelium which were used to calculate the weight of carbon and nitrogen immobilized in the fungal biomass at each sampling time were published by AUSMUS and WITKAMP (1974).

** Aspen leaf litter weight m^{-2} is referenced in PARKINSON and LOUSIER (1974).

Table 3. P, Ca, Mg and K* immobilized in the fungal biomass at each sampling time.

Days from start of study	Month	Nutrient immobilization (mg m^{-2}) in fungal biomass			
		P	Ca	Mg	K
0	Oct.	0.09	1.24	0.07	0.04
4	Oct.	0.10	1.43	0.08	0.05
9	Oct.	0.12	1.68	0.09	0.06
16	Oct.	0.12	1.68	0.09	0.06
19	Oct.	0.35	4.87	0.28	0.18
33	Nov.	0.58	8.03	0.46	0.29
67	Dec.	1.16	16.11	0.92	0.58
102	Jan.	1.18	16.37	0.94	0.59
128	Feb.	1.35	18.69	1.07	0.67
163	Mar.	1.94	26.81	1.53	0.97
186 (during snow-melt)	Apr.	3.41	47.18	2.70	1.70
186 (after snow-melt)	Apr.	1.88	26.08	1.50	0.94

* Concentrations of P, Ca, Mg and K in dry fungal mycelium which were used to calculate the weight of P, Ca, Mg and K immobilized in the fungal biomass at each sampling time were published by AUSMUS and WITKAMP (1974).